International Journal of Agriculture and Wildlife Science (IJAWS), 2020, 6(3): 444 - 452
doi: 10.24180/ijaws.747422

International Journal of Agriculture and Wildlife Science
http://dergipark.org.tr/ijaws

Research Article

Evaluation of Turkish Isolates of Entomopathogenic Fungi Against the Adults of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae)*

Sevim Atmaca[1], Ebubekir Yüksel[2*], Ramazan Canhilal[2]

[1]Department of Plant Protection, Faculty of Agriculture, Bozok University, Yozgat, Turkey
[2]Department of Plant Protection, Faculty of Agriculture, Erciyes University, Kayseri, Turkey

Geliş tarihi (*Received*): 03.06.2020 Kabul tarihi (*Accepted*): 31.08.2020

Keywords:
Stored products, *Sitophilus oryzae*, *Fusarium* spp., *Beauveria bassiana*, *Purpureocillium lilacinum*

***Corresponding author:**
ebubekiryuksel@erciyes.edu.tr

Abstract. In this study, insecticidal effects of 13 Entomopathogenic fungi (EPF) isolates recovered from Adana Province [Nine *Fusarium* spp., three *Beauveria bassiana* (*B. bassiana*) and one *Purpureocillium lilacinum (P. Lilacinum)* isolates] was tested against the adults of *Sitophilus oryzae* (*S. oryzae*) (L.) (Coleoptera: Curculionidae) under controlled conditions at 25±2 °C. Single ($1x10^7$conidia mL^{-1}) and multiple-dose ($1x10^5$, $1x10^6$, $1x10^7$, $1x10^8$ ve $1x10^9$ conidia mL^{-1}) experiments were conducted and mortality rates were recorded on 5^{th}, 7^{th}, 9^{th} and 14^{th} days after treatment (DAT). *P. lilacinum*-224, *B. bassiana*-310 and *Fusarium* sp.-339 were the most efficient isolates in single-dose experiments and included in the multiple-dose bioassay. All the isolates caused high mortality (%100) at both $1x10^8$ and $1x10^9$ doses 14^{th} DAT in the multiple-dose experiment. The calculated LC_{50} and LC_{95} values of the selected isolates were ranged between 889 and 16.231 conidia $adult^{-1}$. The results showed that all isolates tested in multiple-dose experiments have a great potential for the control of the adults of *S. oryzae*. However, further studies are needed to reveal their efficacy under storage conditions.

Yerel Entomopatojen Fungus İzolatlarının *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) Erginleri Üzerindeki Etkinliklerinin Değerlendirilmesi

Anahtar kelimeler:
Depolanmış ürünler, *Sitophilus oryzae*, *Fusarium* spp., *Beauveria bassiana*, *Purpureocillium lilacinum*

Özet. Bu çalışmada, Adana ilinden elde edilen 13 Entomopatojen fungus (EPF) izolatının [dokuz *Fusarium* spp., üç *Beauveria bassiana* (*B. bassiana*) ve bir *Purpureocillium lilacinum (P. Lilacinum)*] insektisit etkisi *Sitophilus oryzae* (*S. oryzae*) (L.) (Coleoptera: Curculionidae) erginlerine karşı kontrollü koşullar altında 25±2 °C'de test edilmiştir. Tekli ($1x10^7$conidia mL^{-1}) ve çoklu doz ($1x10^5$, $1x10^6$, $1x10^7$, $1x10^8$ ve $1x10^9$ conidia mL^{-1}) denemeleri yapılmış ve ölüm oranları uygulamadan sonraki 5., 7., 9. ve 14. günlerde kaydedilmiştir. Tekli doz denemelerinde en etkili izolatlar olarak *P. lilacinum*-224, *B. bassiana* -310 ve *Fusarium* sp.-339 izolatları olmuştur ve bu izolatlar çoklu doz denemelerine dahil edilmişlerdir. Tüm izolatlar, çoklu doz denemelerinde $1x10^8$ ve $1x10^9$ uygulama dozunda uygulamadan sonraki 14. günde yüksek ölüm oranı (% 100) meydana getirmişlerdir. Seçilen izolatların hesaplanan LC_{50} ve LC_{95} değerleri 889 ve 16.231 conidia $ergin^{-1}$ arasında değişmiştir. Sonuçlar, çoklu doz denemelerinde test edilen tüm izolatların, *S. oryzae* erginlerinin kontrolünde büyük bir potansiyele sahip olduğunu göstermiştir. Bununla beraber, bu izolatların depo koşulları altında etkinliklerini belirlenmesi için daha fazla çalışmaya ihtiyaç vardır.

**This article was produced from a master's thesis published in 2016.

ORCID ID (By author order)
0000-0001-8568-3469 0000-0002-6982-5874 0000-0002-0753-0077

INTRODUCTION

Stored product pests can have a large economic impact on stored cereals products and between 5 and 15% of annual post-harvest yield losses occur due to damaging activities of stored product pests (Hagstrum *et al., 1*996). The rice weevil, *Sitophilus oryzae* (L.) (*S. oryzae*) (Coleoptera: Curculionidae), is one of the primary stored-grain insect pests in storage environments and can give rise to great yield losses by decreasing the weight, quality, and commercial value of grains. The control of this pest is quite challenging due to the internal feeding habits of larval stages and reproductive potential of the adults under favorable conditions. Chemicals used in the chemical control of this pest such as phosphine are known to pose serious threats to warm-blooded animals including humans, environment and non-target organisms. Also, the susceptibility of different stages of *S. oryzae* to the chemicals currently in use has been reported to be gradually decreasing (Fields and White, 2002; Boyer *et al.* 2012). Therefore, many researchers are in search of eco-friendly control methods in the control of *S. oryzae* (Lee *et al*., 2001; Athanassiou *et al*., 2004; Ashamo, 2006; Koutsaviti *et al*., 2018; Çolak *et al*., 2019).

Fungal entomopathogens are natural suppressants of insect populations and possess the ability to infect and kill arthropods. Entomopathogenic fungi (EPF) are one of the successful biological control agents of many insect pests and have been studied intensively by many researchers (Lacey *et al.,* 2011; Mnyone *et al.,* 2011; Skinner *et al.,* 2012; Gabarty *et al.,* 2014; Altinok *et al.,* 2019; Canassa *et al.,* 2019). One of the possible usage areas of EPF is the crop storage facilities and screening studies of high-virulent entomopathogenic fungal strains have been accelerated in the last decades (Saranya *et al.,* 2010; Perinotto *et al.,* 2012; Shahid *et al.,* 2012; Cuthbertson and Audsley, 2016; Ausique *et al.,* 2017). Recent studies have revealed the potential of EPF against the stored product pests including *S. oryzae* (Dal Bello *et al.,* 2000; Cherry *et al.,* 2005; Khashaveh *et al.,* 2011; Rumbos and Athanassiou, 2017; Yuksel *et al.,* 2017; Batta, 2018; Ahmed *et al.,* 2019). Although *Beauveria bassiana* is one of the well-studied EPF, control potential of *Purpureocillium lilacinum* and *Fusarium* sp. are less frequently studied in the control of stored product pests.

In the present study, the control potential of thirteen EPF isolates obtained from Adana province (9 *Fusarium* spp., 3 *Beauveria bassiana* and 1 *Purpureocillium lilacinum*) were tested on the adults of *S. oryzae*. Also, the germination rates, spore production and the effect of temperature on mycelial growth and on spore production were determined for the most effective isolates in the pathogenicity tests.

MATERIAL AND METHOD

Source of Insects

The adults of *S. oryzae* were obtianed from the Plant Protection Department of Agricultural Faculty, Erciyes University and cultured in glass jars (1 l volume) with 200 g of wheat at 27±2 °C under dark conditions. The jars were covered with a perforated lid allowing air flow and each jar was observed daily to collect newly emerged adults. Only one-week-old adults (males and females) were used in the experiments.

Fungal Isolates

Nine isolates of *Fusarium* spp., three isolates of *Beauveria bassiana (B. bassiana)* and one isolate of *Purpureocillium lilacinum (P. Lilacinum)* were used in the experiments (Table 2). All the isolates tested were recovered from Adana province (Turkey). Morphological identification of fungal isolates was made under a microscope according to the criteria suggested by Humber (1997) and verified by Dr. Richard Humber (United States Department of Agriculture, Agricultural Research Service). The isolates were grown on PDA in 9 cm diameter Petri dishes and maintained in darkness at 25±2 °C for 14 days for the completion of sporulation. Fungal conidia were concentrated by scraping from the conidial layer using a sterile scalpel and harvested by flooding the Petri dishes with sterile distilled water containing 0.01% (v/v) Tween 20 (Sigma Chemical, St. Louis, Mo, USA) and shaking with a glass rod. The conidial concentration was calculated with a hemocytometer and set up to 7×10^6 conidia mL^{-1} after the filtration of the suspension through a sieve (60-mesh) to separate hyphae and substrate.

Bioassays

A single-dose ($1x10^7$ conidia mL^1) experiment was carried out in Petri dishes (9 cm diameter) including filter paper to evaluate the pathogenicity of each isolate. Randomly selected twenty *S. oryzae* adults were put into each Petri dish and 1 mL of aqueous conidial suspension was added with the help of a sterile perfume sprayer. The adults from each treatment were put into untreated Petri dishes containing 10 g of intact wheat after kept

for 24 h post-inoculation without food at 25±2 °C, 70±5% RH. The Petri dishes were observed at 5th, 7th, 9th, and 14th days after treatment (DAT) to distinguish the dead individuals (Dal Bello *et al*., 2000; Kavallieratos, 2014). Each treatment replicated four times for each isolate and only Tween 20 in distilled water (0.01%) was applied to control treatments. The dead adults were surface-sterilized in 1% sodium hypochlorite solution, washed 3 times with sterile water and then placed on moistened filter paper. The adults with the hyphal growth characteristics were counted as fungi-infected after the cadavers were kept in darkness for one week at 25±2 °C (Ali-Shtayeh, 2003).

In the multiple-dose experiments five conidial concentrations (1x10^5, 1x10^6, 1x10^7, 1x10^8, and 1x10^9 conidia mL^{-1}) of the most effective three isolate was evaluated on twenty adults of *S. oryzae*. The mortality rates of the adults were recorded on the 5th, 7th, 9th, and 14th and the infection of isolates were confirmed following the aforementioned procedure. Mycosis values of EPF on *S. oryzae* were calculated by the ratio of adults. Suspensions of 0.5% (w/w) of formulations or 0.1% (w/w) of unformulated conidia in sterile distilled water were prepared and then diluted 100-fold. Twenty-five microliters were gathered from the above diluted suspensions then spread in a thin layer on the surface of glass slides kept in Petri dishes under humid conditions at 25±2 ºC for 24 h. The germinations of conidia were evaluated 24 h post-inoculation by calculating germinated and nongerminated conidia under the microscope (400X). The average germination percentage of the counted conidia was calculated for each sample. Total germination percentage is calculated by counting 100 spores from each sample. Germ tube spores are widely regarded as germinated spores. Spore suspension was applied to the adults by spray inoculation method set to 1x10^6 mL^{-1} and the weevils were kept at 25±2 ºC for 7 days. The dead insects have been harvested for hydration for sporulation. Spore production was determined afterwards. To determine this, a total of 15 infectious insects (5 infectious insects for each repeat) were thrown separately into sterile 0.01% Tween 20 solution, with 3 replicates for each fungus. The numbers of spores were determined by hemocytometer after it was vortexed for a minute to facilitate the passage of spores into the water. Media were dispensed in 90 mm diameter Petri dishes and were centrally inoculated with 6 mm agar plugs from 7-day-old PDA cultures of fungal isolates. Three replicate dishes of each medium for each fungal isolate were incubated for 7 days at 10, 15, 20, 25, 30, and 37 ºC in darkness. Radial mycelial growth was then recorded as the mean of two perpendicular radii minus the radius of the inoculum plug. Radial mycelial growth rate was calculated in mm/day. Spores production tested for six temperatures including 10, 15, 20, 25, 30, and 37 ºC with three replicates/temperature combination on PDA supplemented. At the end of the mycelial growth study (14th day) spores were harvested by flooding the plates with 10 mL of distilled water and scraping the surface of the colonies with a glass slide. The suspensions were filtered through a layer of sterile tissue papers and spore concentration in the suspension from three colonies/temperature was determined using a hemocytometer.

Statistical Analysis

In the pathogenicity bioassays, mortality data set were subjected to analysis of variance (ANOVA) using SPSS (2013) after checked for normal distribution and adjusted using arcsine transformation. Means were separated by using the Tukey test ($P \leq 0.05$). Lethal concentration values (LC_{50} and LC_{95}) of each isolate was determined using Probit analysis.

RESULTS AND DISCUSSION

In order to obtain EPF, 80 soil samples in total were taken from different habitats of Adana Province and thirteen of them were found positive (16.2%). Nine isolates were identified as *Fusarium* sp., three were *Beauveria bassiana*, and one was *Purpureocillium lilacinum*. EPF were isolated from all habitats soil samples taken and Orchard/Vineyard habitats showed a higher recovery frequency (22%) than the others (Table 1). All the isolates caused great mortality on the adults of *S. oryzae* varying between 47% and 98% at 14th DAT and fungal species showed great variability in virulence against the adults of *S. oryzae* (Table 2). Mortality rates generally increased as the DAT increased in the single-dose experiment. *Beauveria bassiana*-310 and *Fusarium* sp.-339 were the most efficacious isolates at 5th-7th DAT and at 9th-14th DAT, respectively. Purpureocillium lilacinum-224 performed better than the most of the isolates at the days after application (Table 3). There was no mortality in the control treatments. Post-mortem mycelial and conidial growth confirmed that most of the adults died of the EPF application. The external mycelium emerged within 8 days after keeping the dead adults on a damp filter paper. The highest rate of mycosis (35%) was achieved by *B. bassiana*-310 isolate at the 7th and 14th DAT (Table 4). Three most virulent isolates were selected after the single-dose experiment and included in

the multiple-dose experiment (*B. bassiana*-310 isolate, *Fusarium* sp.-339 isolate, and *P. lilacinum*-224 isolate) (Table 5, 6, 7, 8).

Table 1. Distribution of EPF in soil by geographical location, crop, and percentage frequency of isolates.
Çizelge 1. Entomopatojen fungus izolatlarının izole edildikleri coğrafi konumu, tarımsal ürün ve izolasyon yüzdesine göre dağılımı.

		Habitats (Number of positive samples/ Number of samples tested)*								
Sample code	**Location**	**Pasture**	**Vegetable**	**Orchard/ vineyard**	**Cropland**	**Forest**	**% F**	**% B**	**% P**	**% EPF**
165	Bahçeköy-Karataş	0/1	1(F)/4	0/1	0/3	-	11.1	0.0	0.0	11.1
195-213	Ceyhan	1(B)/5	-	1(F)/7	0/11	0/2	4.0	4.0	0.0	8.0
224	Mansurlu -Kozan	-	-	1(P)/1	0/1	-	0.0	0.0	50.0	50.0
251-252	Karaömerli-Sarıçam	1(F)/1	-	1(F)/1	0/1	-	66.7	0.0	0.0	66.7
307-310	Çukurova	0/1	1(B)/4	1(B)/5	0/4	0/2	0.0	12.5	0.0	12.5
327-328-334	Karaisalı	1(F)/3	0/2	0/2	0/5	2(F)/6	16.7	0.0	0.0	16.7
339	Feke	-	-	1(F)/5	0/1	-	16.7	0.0	0.0	16.7
452	Kahramanmaraş, Afşin, Alipınar	-	-	-	1(F)/1	-	100.0	0.0	0.0	100.0

*F: *Fusarium* spp., B: *Beauveria bassiana,* P: *Purpureocillium lilacinum*.

Table 2. MANOVA parameters for the main effects and associated interactions for the mortality rates of the adults *Sitophilus oryzae*.
Çizelge 2. Sitophilus oryzae erginlerinin ölüm oranları için ana faktörler ve interaksiyonlarının çok değişkenli varyans analizi (MANOVA).

Source*	**5th DAT**			**7th DAT**			**9th DAT**			**14th DAT**		
	df	**F**	**P**	**df**	**F**	**P**	**df**	**F**	**P**	**df**	**F**	**P**
F	2	41.976	**0.000**	2	35.670	**0.000**	2	56.078	**0.000**	2	69.392	**0.000**
C	4	127.449	**0.000**	4	199.269	**0.000**	4	299.428	**0.000**	4	406.465	**0.000**
F*C	8	2.503	**0.024**	4	2.097	0.056	8	1.703	0.124	8	20.902	**0.000**

*DAT: Days after EPF-treatment, F: Entomopathogenic fungi isolates, C: Concentrations, F: F-statistic, P: Probability for significant level and df: the degree of freedom. *Calculated P-value ≤ 0.05 indicates that the source of variation in mortality of adult *S.oryzae* was significant.

Table 3. Mortality rates of thirteen EPF isolates on the adults of *Sitophilus oryzae* at the 5th, 7th, 9th and 14th days after treatment (DAT) in the single-dose experiment (1x10^7 conidia mL^{-1}) at 25±2°C.
Çizelge 3. On üç entomopatojen fungus izolatının tek doz denemelerinde (1x10^7 conidia mL^{-1}) 25 ± 2 °C'de uygulamadan sonraki 5., 7., 9. ve 14. günlerde Sitophilus oryzae erginleri üzerinde meydana getirdikleri ölüm oranları.

EPF		**Mortality Rates (±Se)**			
	Isolate	**5th DAT**	**7th DAT**	**9th DAT**	**14th DAT**
Beauveria bassiana	195	20.0±3.1Bc*	35.0±4.5CDb	68.8±3.7DEa	69.0±3.7DEa
Beauveria bassiana	307	12.5±2.8BCDc	51.3±5.2BCb	83.8±4.1ABCa	85.0±4.1ABCa
Beauveria bassiana	310	35.0±3.1Ac	75.0±3.1Ab	92.5±2.8ABa	94.3±2.8ABa
Fusarium sp.	165	7.5±1.3DEb	35.0±5.3CDa	47.5±4.2Fa	47.5±4.2Fa
Fusarium sp.	213	6.3±2.1Ec	23.8±2.1Db	63.8±5.8Ea	65.0±4.8Ea
Fusarium sp.	251	6.3±3.3Ec	40.0±4.4CDb	72.5±4.5CDEa	73.0±4.5CDEa
Fusarium sp.	252	6.3±1.1Ec	35.0±5.3CDb	61.3±5.7Ea	65.0±5.6Ea
Fusarium sp.	327	17.5±3.8BCc	52.5±1.3BCb	72.5±3.7CDEa	72.5±3.8CDEa
Fusarium sp.	328	8.8±3.7CDEc	46.3±4.2Cb	80.0±4.3BCDa	82.3±4.2BCDa
Fusarium sp.	334	11.3±2.1BCDc	36.3±3.1BCb	83.8±4.5ABCa	83.8±4.5BCDa
Fusarium sp.	339	18.8±4.1Bc	71.3±7.2Ab	97.5±2.1Aa	98.8±2.2Aa
Fusarium sp.	452	13.8±3.7BCDc	65.0±4.3ABb	90.0±3.5ABa	90.3±3.5ABa
Purpureocillium lilacinum	224	21.3±2.1Bc	67.5±4.2ABb	90.0±1.8ABa	90.5±1.8ABa
Control	-	0.0±0.0Fa	3.8±2.1Ea	6.3±2.1Ga	6.3±2.1Ga

*Mean values followed by different uppercase letters in the same column and mean values followed by different lowercase letters in the same line are statistically different according to Tukey's test ($P \leq 0.05$).

The estimated LC_{50} values of the isolates tested on the adults of *S. oryzae* were given in Table 8. The lowest LC_{50} and LC_{95} values were achieved by *Fusarium* sp.-339 isolate. The spore production of the isolates ranged between $0.70x10^2$ and $5.20x10^3$ (Table 9).

Many researchers have studied the effectiveness of EPF against the adults of *S. oryzae* using different application methods and concentrations. The susceptibility of the adults was varied according to the EPF species, the virulence of EPF species and isolates, and the method of treatment or application. Dal Bello *et al.* (2000) evaluated the virulence of ten different fungal isolates belonging to *B. bassiana*, *M. anisopliae*, *Verticillium lecanii*, and *P. farinosus* against the adults of *S. oryzae* and the highest mortality rates did not exceed 50% 14th DAT. Rice and Cogburn (1999) reported 100% adult mortality of *S. oryzae* 14*th* DAT when *B. bassiana* isolate applied as a conidial powder. In another study, the maximum mortality rate (100%) achieved from the conidial suspension of *B. bassiana* 7th DAT (Kavallieratos *et al.,* 2014). Mortality rates generally increased in these studies with the increasing concentrations. Our study is in agreement with these reports that higher mortality obtained with increasing concentrations. This may be originated from the low concentrations that may not be able to break the resistance mechanisms of healthy adults. Contrary to this, the adults were not able to get over or survive the infection in high concentrations (Muller-Kogler, 1967; Makaka, 2008).

The type of formulation used, the virulence of isolates, environmental conditions, and concentration of conidia are also crucial to understand the considerable variation in susceptibility of the adults (Wakefield, 2006). Some of the isolates used in this study were found to work well at 25 °C and 70±5% RH and caused high mortality by the direct inoculation of adults with the conidial suspension of the EPF isolates. The efficacy of different EPF species on the adults of *S. oryzae* has been studied by various researches worldwide and *B. bassiana* provided effective control in these studies (Hluchý and Samšiňáková, 1989; Rice and Cogburn, 1999; Sheeba *et al*., 2001; Yanar *et al*., 2019; Yanar *et al*., 2020).

Table 4. Mycosis rates (%) of thirteen EPF isolates on the adults of *Sitophilus oryzae* at the 7th and 14th days after treatment (DAT) after the single-dose experiment ($1x10^7$ conidia mL^{-1}) at 25±2 °C.

Çizelge 4. On üç entomopatojen fungus izolatının (%), tek doz denemelerinde ($1x10^7$ conidia mL^{-1}) uygulamadan sonraki 7. ve 14. günlerde 25±2 °C'de Sitophilus oryzae erginleri üzerindeki mikozis oranları.

EPF	Isolates	Days after treatment (DAT) (Mycosis %)	
		7th	14th
Beauveria bassiana	195	20.0 Ab*	35.0 Ca
Beauveria bassiana	307	12.5 BCb	51.3 Ba
Beauveria bassiana	310	35.0 Ab	75.0 Aa
Fusarium sp.	165	7.5 Cb	35.0 Ca
Fusarium sp.	213	6.3 Cb	23.8 CDa
Fusarium sp.	251	6.3 Cb	40.0 Ca
Fusarium sp.	252	6.3 Cb	35.0 Ca
Fusarium sp.	327	17.5 Bb	52.5 Ba
Fusarium sp.	328	8.8 Cb	46.3 BCa
Fusarium sp.	334	11.3 BCb	36.3 Ca
Fusarium sp.	339	18.8 ABb	71.3 Aa
Fusarium sp.	452	13.8 BCb	65.0 Aa
Purpureocillium lilacinum	224	21.3 Ab	67.5 Aa
Control	-	0.0 Da	0.0 Da

*Mean values followed by different uppercase letters in the same column and mean values followed by different lowercase letters in the same line are statistically different according to Tukey's test ($P \leq 0.05$).

Rapid germination and penetration of the conidia are the crucial factors affecting the speed of kill and play a key role in the virulence of EPF species as indicated in earlier studies (Altre and Vandenburg, 2001; Wakefield, 2006). In this study, early penetration of *Fusarium* spp. and *B. bassiana* into the insect tissue is accompanied by the rapid death of the adults of *S. oryzae*. In this study, LC_{50} and LC_{95} values of *B. bassiana*-310 and *Fusarium* sp.-339 were quite similar to each other and below the earlier reported ranges for different pests (Sheeba *et al*., 2001; Batta, 2010; Mahdneshin *et al*., 2011).

Table 5. Dose mortality rates (%) of *P. lilacinum* 224, *B. bassiana* 310 and *Fusairum* sp. 339 entomopathogenic fungi isolates on the adults of *Sitophilus oryzae* at the 5th days after treatment (DAT).

Çizelge 5. Purpureocillium lilacinum 224, B. bassiana 310 and Fusairum sp. 339 entomopatojen fungus izolatlarının doz çalışmasında uygulamadan sonraki 5. günde Sitophilus oryzae erginleri üzerinde meydana getirdikleri ölüm oranları.

EPF	Isolates	Dose (Conidi mL^{-1}) ± Se				
		1x10^5	1x10^6	1x10^7	1x10^8	1x10^9
Purpureocillium lilacinum	224	2.5±2.2Bc*	10.0±3.4ABc	27.5±3.8Bb	42.5±1.3Ba	48.8±2.1Ca
Beauveria bassiana	310	15.0±3.1ABc	18.8±3.3ABc	40.0±3.1Ab	50.0±0.0Bb	66.3±4.5Ba
Fusarium sp.	339	15.0±3.4ABd	25.5±2.2Ad	45.0±1.8Ac	68.8±5.1Ab	85.0±3.1Aa
Control	-	0.0±0.0Ba	1.3±1.1Ba	1.3±1.1Ca	0.0±0.0Ca	2.5±1.3Da

*Mean values followed by different uppercase letters in the same column and mean values followed by different lowercase letters in the same line are statistically different according to Tukey's test ($P \leq 0.05$).

Table 6. Dose mortality rates (%) of *P. lilacinum* 224, *B. bassiana* 310 and *Fusairum* sp. 339 EPF isolates on the adults of *Sitophilus oryzae* at the 7th DAT.

Çizelge 6. Purpureocillium lilacinum 224, B. bassiana 310 and Fusairum sp. 339 entomopatojen fungus izolatlarının doz çalışmasında uygulamadan sonraki 7. günde Sitophilus oryzae erginleri üzerinde meydana getirdikleri ölüm oranları.

EPF	Isolate	Dose (Conidi mL^{-1}) ± Se				
		1x10^5	1x10^6	1x10^7	1x10^8	1x10^9
Purpureocillium lilacinum	224	8.8±2.8ABd*	30.0±1.8BCc	41.3±5.4Cb	72.5±1.3Ba	65.0±1.8Ba
Beauveria bassiana	310	21.3±1.1Ad	41.3±3.7Ac	52.5±1.3Bb	82.5±2.2Aa	81.3±2.1Aa
Fusarium sp.	339	22.5±2.2Ad	37.5±2.8ABc	68.8±5.1Ab	85.0±3.1Aa	90.0±2.5Aa
Control	-	1.3±1.1Ba	0.0±0.0Ca	0.0±0.0Da	0.0±0.0Ca	2.5±1.3Ca

*Mean values followed by different uppercase letters in the same column and mean values followed by different lowercase letters in the same line are statistically different according to Tukey's test ($P \leq 0.05$).

Table 7. Dose mortality rates (%) of *P. lilacinum* 224, *B. bassiana* 310 and *Fusairum* sp. 339 EPF isolates on the adults of *Sitophilus oryzae* at the 9th DAT.

Çizelge 7. Purpureocillium lilacinum 224, B. bassiana 310 and Fusairum sp. 339 entomopatojen fungus izolatlarının doz çalışmasında uygulamadan sonraki 9. günde Sitophilus oryzae erginleri üzerinde meydana getirdikleri ölüm oranları.

EPF	Isolate	Dose (Conidi mL^{-1}) ± Se				
		1x10^5	1x10^6	1x10^7	1x10^8	1x10^9
Purpureocillium lilacinum	224	21.3±2.1Bd*	43.8±5.4Bc	58.8±3.7Cb	85.0±0.0Ba	77.5±1.3Ba
Beauveria bassiana	310	35.0±1.8Ad	60.0±3.1Ac	90.0±3.1Ab	98.8±1.1Aa	100.0±0.0Aa
Fusarium sp.	339	33.8±2.1Ad	58.8±3.7Ac	77.5±2.8Bb	95.0±1.8Aa	97.5±2.2Aa
Control	-	1.3±1.1Ca	1.3±1.1Ca	1.3±1.1Da	1.3±1.1Ca	2.5±1.Ca

*Mean values followed by different uppercase letters in the same column and mean values followed by different lowercase letters in the same line are statistically different according to Tukey's test ($P \leq 0.05$).

Table 8. Dose mortality rates (%) of *P. lilacinum* 224, *B. bassiana* 310 and *Fusairum* sp. 339 EPF isolates on the adults of *Sitophilus oryzae* at the 14th DAT.

Çizelge 8. Purpureocillium lilacinum 224, B. bassiana 310 and Fusairum sp. 339 entomopatojen fungus izolatlarının doz çalışmasında uygulamadan sonraki 14. günde Sitophilus oryzae erginleri üzerinde meydana getirdikleri ölüm oranları.

EPF	Isolate	Dose (Conidi mL^{-1}) ± Se				
		1x10^5	1x10^6	1x10^7	1x10^8	1x10^9
Purpureocillium lilacinum	224	30.0±2.5Cd*	52.5±1.3Cc	73.5±4.5Cb	100.0±0.0Aa	100.0±0.0Aa
Beauveria bassiana	310	47.5±2.2Ac	72.5±2.2Bb	100.0±0.0Aa	100.0±0.0Aa	100.0±0.0Aa
Fusarium sp.	339	45.0±0.0ABc	93.8±3.3Ab	90.0±1.8Bb	100.0±0.0Aa	100.0±0.0Aa
Control	-	1.3±1.1Da	1.3±1.1Da	1.3±1.1Da	5.0±2.5Ba	2.5±1.3Ba

*Mean values followed by different uppercase letters in the same column and mean values followed by different lowercase letters in the same line are statistically different according to Tukey's test ($P \leq 0.05$).

Table 9. Lethal concentration 50% (LC_{50}) ve 95% (LC_{95}) values (Conidia mL^{-1}) of the adults of *Sitophilus oryzae* ($P \leq 0.05$).
Çizelge 9. Sitophilus oryzae erginlerinin lethal konsantrasyon 50% (LC_{50}) ve 95% (LC_{95}) değerleri (Conidia mL^{-1}) ($P \leq 0.05$).

EPF	Isolate	LC_{50}	LC_{95}	X^2	P	Slop (b)	Intercept (a)
Purpureocillium lilacinum	224	8892.5	16230.7	0.69	0.92	1.42	-2.02
Beauveria bassiana	310	4863.4	10153.9	3.74	0.98	2.07	-0.39
Fusarium sp.	339	2011.6	10055.4	3.66	0.28	2.34	-1.31

CONCLUSION

These results indicate that *B. bassiana*-310 and *Fusarium* sp.-339 could significantly help to minimize the damages caused by the adults of *S. oryzae* in storage environment under favorable conditions. Synergistic studies with entomopathogenic nematodes and biopesticides are highly recommended for further studies in an empty storage environment and could help achieve better control against the adults of *S. oryzae*.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTIONS

SA and RC conceived and designed the research. SA conducted the experiments. SA and EY analyzed the data and wrote the manuscript. All authors read and approved the manuscript.

ACKNOWLEDGMENT

This study was supported by the office of scientific research projects of Erciyes University (Project No. FYL-2014-5465). The funders had no role in the design of the study and collection, analysis, interpretation of data, and in writing the manuscript.

REFERENCES

Ahmed, N., Lahori, A. H., Feng, J. N., & Wang, D. (2019). Use of indigenous isolates of *Metarhizium, Isaria* and *Beauveria* as potential bio-control agents against *Sitophilus oryzae* under laboratory conditions. *Pakistan Journal of Agricultural Sciences, 56*(4), 1037-1044.

Ali-Shtayeh, M. S., Mara'i, A. B. B., & Jamous, R. M. (2003). Distribution, occurrence and characterization of entomopathogenic fungi in agricultural soil in the Palestinian area. *Mycopathologia, 156*(3), 235-244.

Altinok, H. H., Altinok, M. A., & Koca, A. S. (2019). Modes of action of entomopathogenic fungi. *Current Trends in Natural Sciences, 8*(16), 117-124.

Altre, J. A., & Vandenberg, J. D. (2001). Penetration of cuticle and proliferation in hemolymph by *Paecilomyces fumosoroseus* isolates that differ in virulence against lepidopteran larvae. *Journal of Invertebrate Pathology, 78*(2), 81-86.

Ashamo, M. O. (2006). Relative susceptibility of some local and elite rice varieties to the rice weevil, *Sitophilus oryzae* L.(Coleoptera: Curculionidae). *Journal of Food Agriculture and Environment, 4*(1), 249.

Ausique, J. J. S., D'Alessandro, C. P., Conceschi, M. R., Mascarin, G. M., & Júnior, I. D. (2017). Efficacy of entomopathogenic fungi against adult *Diaphorina citri* from laboratory to field applications. *Journal of Pest Science*, *90*(3), 947-960.

Athanassiou, C. G., Papagregoriou, A. S., & Buchelos, C. T. (2004). Insecticidal and residual effect of three pyrethroids against *Sitophilus oryzae* (L.)(Coleoptera: Curculionidae) on stored wheat. *Journal of Stored Products Research, 40*(3), 289-297.

Batta, Y., Murdoch, G., & Mansfield, S. (2010). Investigations into the formulation and efficacy of entomopathogenic fungi against larvae of yellow mealworm (*Tenebrio molitor* L., Coleoptera: Tenebrionidae). *General and Applied Entomology, 39*, 5-8.

Batta, Y. A. (2018). Efficacy of two species of entomopathogenic fungi against the stored-grain pest, *Sitophilus granarius* L.(Curculionidae: Coleoptera), via oral ingestion. *Egyptian Journal of Biological Pest Control*, *28*(1), 44.

Boyer, S., Zhang, H., & Lempérière, G. (2012). A review of control methods and resistance mechanisms in stored-product insects. *Bulletin of Entomological Research, 102*(2), 213-229.

Canassa, F., Tall, S., Moral, R. A., de Lara, I. A., Delalibera Jr, I., & Meyling, N. V. (2019). Effects of bean seed treatment by the entomopathogenic fungi *Metarhizium robertsii* and *Beauveria bassiana* on plant growth, spider mite populations and behavior of predatory mites. *Biological Control*, *132*, 199-208.

Cherry, A. J., Abalo, P., & Hell, K. (2005). A laboratory assessment of the potential of different strains of the entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) to control *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in stored cowpea. *Journal of Stored Products Research, 41*(3), 295-309.

Cuthbertson, A. G., & Audsley, N. (2016). Further screening of entomopathogenic fungi and nematodes as control agents for *Drosophila suzukii*. *Insects*, *7*(2), 24.

Çolak, E. Ş., Yüksel, E., & Canhilal, R. (2019) Yabancı Otların Kontrolünde Biyolojik Mücadele. *Erciyes Tarım ve Hayvan Bilimleri Dergisi, 2*(3), 23-29.

Dal Bello, G., Padin, S., Lastra, C. L., & Fabrizio, M. (2000). Laboratory evaluation of chemical-biological control of the rice weevil (*Sitophilus oryzae* L.) in stored grains. *Journal of Stored Products Research, 37*(1), 77-84.

Fields, P. G., & White, N. D. (2002). Alternatives to methyl bromide treatments for stored-product and quarantine insects. *Annual Review of Entomology, 47*(1), 331-359.

Gabarty, A., Salem, H. M., Fouda, M. A., Abas, A. A., & Ibrahim, A. A. (2014). Pathogencity induced by the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in *Agrotis ipsilon* (Hufn.). *Journal of Radiation Research and Applied Sciences*, *7*(1), 95-100.

Hagstrum, D. W., Flinn, P. W., & Shuman, D. (1996). Automated monitoring using acoustical sensors for insects in farm-stored wheat. *Journal of Economic Entomology, 89*(1), 211-217.

Hluchý, M., & Samšiňáková, A. (1989). Comparative study on the susceptibility of adult *Sitophilus granarius* (L.)(Coleoptera: Curculionidae) and larval *Galleria mellonella* (L.)(Lepidoptera: Pyralidae) to the entomogenous fungus *Beauveria bassiana* (Bals.) Vuill. *Journal of Stored Products Research, 25*(2), 61-64.

Humber, R. A. (1997). Fungi: identification. In L. Lacey (Ed), *Manual of techniques in insect pathology* (pp. 153-185). San Dieogo, USA: Academic Press.

Kavallieratos, N. G., Athanassiou, C. G., Aountala, M. M., & Kontodimas, D. C. (2014). Evaluation of the entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae*, and *Isaria fumosorosea* for control of *Sitophilus oryzae*. *Journal of Food Protection, 77*(1), 87-93.

Khashaveh, A., Ghosta, Y., Safaralizadeh, M. H., & Ziaee, M. (2011). The use of entomopathogenic fungus, *Beauveria bassiana* (Bals.) Vuill. in assays with storage grain beetles. *Journal Of Agricultural Science And Technology, 13*(1), 35-42.

Koutsaviti, A., Antonopoulou, V., Vlassi, A., Antonatos, S., Michaelakis, A., Papachristos, D. P., & Tzakou, O. (2018). Chemical composition and fumigant activity of essential oils from six plant families against *Sitophilus oryzae* (Col: Curculionidae). *Journal of Pest Science, 91*(2), 873-886.

Lacey, L. A., Liu, T. X., Buchman, J. L., Munyaneza, J. E., Goolsby, J. A., & Horton, D. R. (2011). Entomopathogenic fungi (Hypocreales) for control of potato psyllid, *Bactericera cockerelli* (Šulc)(Hemiptera: Triozidae) in an area endemic for zebra chip disease of potato. *Biological Control*, *56*(3), 271-278.

Lee, B. H., Choi, W. S., Lee, S. E., & Park, B. S. (2001). Fumigant toxicity of essential oils and their constituent compounds towards the rice weevil, *Sitophilus oryzae* (L.). *Crop Protection, 20*(4), 317-320.

Mahdneshin, Z., Vojoudi, S., Ghosta, Y., Safaralizadae, M. H., & Saber, M. (2011). Laboratory evaluation of the entomopathogenic fungi, Iranian isolates of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metsch) Sorokin against the control of the cowpea weevil, *Callosobruchus maculatus* F. (Coleoptera: Bruchidae). *African Journal of Microbiology Research, 5*, 5215-5220.

Makaka, C. (2008). The efficacy of two isolates of *Metarhizium anisopliae* (Metschin) Sorokin (Deuteromycotina: Hyphomycetes) against the adults of the black maize beetle *Heteronychus licas* Klug (Coleoptera: Scarabidae) under laboratory conditions. *African Journal of Microbiology Research*, *3*(4), 259-265.

Mnyone, L. L., Kirby, M. J., Mpingwa, M. W., Lwetoijera, D. W., Knols, B. G., Takken, W., & Russell, T. L. (2011). Infection of *Anopheles gambiae* mosquitoes with entomopathogenic fungi: effect of host age and blood-feeding status. *Parasitology Research*, *108*(2), 317-322.

Müller-Kögler, E. (1967). On mass cultivation, determination of effectiveness, and standardization of insect pathogenic fungi. *Insect Pathology and Microbial Control*, 339-353.

Perinotto, W. M. S., Angelo, I. C., Golo, P. S., Quinelato, S., Camargo, M. G., Sá, F. A., & Bittencourt, V. R. E. P. (2012). Susceptibility of different populations of ticks to entomopathogenic fungi. *Experimental Parasitology*, *130*(3), 257-260.

Rice, W. C., & Cogburn, R. R. (1999). Activity of the entomopathogenic fungus *Beauveria bassiana* (Deuteromycota: Hyphomycetes) against three coleopteran pests of stored grain. *Journal of Economic Entomology, 92*(3), 691-694.

Rumbos, C. I., & Athanassiou, C. G. (2017). Use of entomopathogenic fungi for the control of stored-product insects: can fungi protect durable commodities?. *Journal of Pest Science*, *90*(3), 839-854.

Saranya, S., Ushakumari, R., Jacob, S., & Philip, B. M. (2010). Efficacy of different entomopathogenic fungi against cowpea aphid, *Aphis craccivora* (Koch). *Journal of Biopesticides*, *3*, 138.

Shahid, A. A., Rao, Q. A., Bakhsh, A., & Husnain, T. (2012). Entomopathogenic fungi as biological controllers: new insights into their virulence and pathogenicity. *Archives of Biological Sciences*, *64*(1), 21-42.

Sheeba, G., Seshadri, S., Raja, N., Janarthanan, S., & Ignacimuthu, S. (2001). Efficacy of *Beauveria bassiana* for control of the rice weevil *Sitophilus oryzae* (L.)(Coleoptera: Curculionidae). *Applied Entomology and Zoology, 36*(1), 117-120.

Skinner, M., Gouli, S., Frank, C. E., Parker, B. L., & Kim, J. S. (2012). Management of *Frankliniella occidentalis* (Thysanoptera: Thripidae) with granular formulations of entomopathogenic fungi. *Biological Control*, *63*(3), 246-252.

SPSS. (2013). IBM SPSS Statistics 21.0 for Windows. Armonk, NY.

Wakefield, M. E. (2006). *Factors affecting storage insect susceptibility to the entomopathogenic fungus Beauveria bassiana*. 9th International Working Conference on Product Protection, São Paulo.

Yanar, Y., Yanar, D., Demir, B., & Karan, Y. B. (2019). Effects of local entomopathogenic *Beauveria bassiana* isolates against *Sitophilus granarius* (Coleoptera). *Poljoprivredai Sumarstvo, 65*(1), 49-55.

Yanar, Y., Yanar, D., & Budak, D. S. (2020). Effects of *Beauveria bassiana* isolates on *Sitophilus oryzae* under in vitro conditions. *Fresenius environmental bulletin*, 3422.

Yüksel, E., Açıkgöz, Ç., Demirci, F., & Muştu, M. (2017). Effects of the entomopathogenic fungi, *Beauveria bassiana*, *Isaria farinosa* and *Purpureocillium lilacinum*, on eggs of *Tuta absoluta* (Meyrick)(Lepidoptera: Gelechiidae). *Türkiye Biyolojik Mücadele Dergisi, 8*(1), 39-47.